A STUDY OF OXYGEN METABOLISM IN *DROSOPHILA MELANOGASTER.*

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Introduction.

Although *Drosophila melanogaster* is uniquely favorable as material for genetical studies, relatively few physiological investigations have been conducted upon this animal. Its small size has certainly been a deterrent to such studies; yet, with suitable apparatus, this feature is unimportant. To supplement the remarkably full record we possess for its genetical behavior physiological studies are especially desirable, and an attempt is made in this paper to show the practicability of investigations into the metabolism of this fly and also to illustrate the type of results which such study can be expected to yield. The investigation was undertaken primarily to determine to what extent degree of inbreeding may be reflected in metabolism.

Measurements were made of the oxygen consumption of *Drosophila* pupæ, and these proved admirably adapted to this purpose. At no time during pupal life except at its extreme termination are results complicated by muscular movements, hence standard metabolism alone is measured. Moreover, the fly is so amenable to conditions of laboratory culture that experimental pupæ are available at all times and can be grown under standard conditions.

The investigation was conducted at the University of Pennsylvania, for which privilege the writer desires to acknowledge his indebtedness to Doctor C. E. McClung. He is under special obligations to Doctor J. H. Bodine, who not only suggested the problem but was ever ready with helpful suggestions throughout the progress of the work. The stocks of experimental flies were kindly contributed by Doctors C. B. Bridges, H. J. Muller, L. E. Griffin, Chas. Zeleny, J. H. Bodine and R. L. King.

Material and Methods.

Eight stocks of "wild" *Drosophila melanogaster* were employed in the study. Three of these were caught shortly before the

work was undertaken in localities removed from centers where flies are grown. These may be called the "non-inbreds." The remaining five stocks had been inbred for a number of years before being received and, as some of these are well known, the following table of sources may be of interest.

TABLE I.

SHOWING DERIVATION OF EXPERIMENTAL MATERIALS.

NON-INBREDS.

Designation.	Source.	Captured.
G.....	Portland, Oregon	August, 1923
H.....	Hellam, Penna.	July, 1923
B.....	Rockaway, N. J.	July, 1923

INBREDS.

Designation.	Original Name.	Source.	Inbred Since
C–2.....	"Ossining"	Columbia University	1921
C–1.....	"Pt. Pleasant"	Columbia University	1921
F.....	"Florida No. 5"	University of Texas	1918
I.....	——	University of Illinois	1916
P.....		University of Penna.	Many years

Owing to some preliminary difficulties, work was not begun until December, 1923, or until the non-inbred stocks had become inbred for several generations. Thereafter readings were continued, with interruptions, until December, 1924, when the experimental work was concluded. The chronological distribution of the work is without significance to our study and will not be entered into.

The pupæ of *Drosophila melanogaster* are so small that it is impracticable to make an extensive series of measurements of oxygen consumption on single pupæ, hence lots of 10 or fewer pupæ were used for each determination. Readings were taken over a period of 4 or 5 hours each day throughout the duration of pupal life. Data were collected for 160 lots of pupæ.

The stock flies were grown in mass culture in large quinine bottles and kept at room temperature. The experimental pupæ, however, were always the products of single matings. From time to time the flies in a culture bottle were removed and matings were made up from new flies as they appeared which

were never more than 20 hours old. The pairs of flies were cultured in shell vials (about 9 cm. long by 2 cm. diameter) containing banana agar and were transferred each day to fresh vials so that the pupæ forming in a particular vial resulted from eggs deposited therein on a single and known day. A complete cultural record was kept which included figures for the sex ratios of the flies appearing in all of the vials in order that a check might be had on any conditions of metabolism attributable to sex peculiarities of the matings. It happened, however, that for all of the matings the distribution of the sexes remained normal. Some of the matings were cultured at variable room temperature, ranging from 21° C. to 25° C. about a mean of 23° C.; others were cultured in an incubator at a constant temperature of 25° C. It is necessary to stress this distinction, for upon it will be based a natural division of the data into two parts. Hereafter, the pupæ formed at room temperature will be referred to as of the "first experimental period," whereas those formed at 25° C. will be referred to as of the "second experimental period."

The banana-agar was prepared according to the usual method and while still liquid about 5 or 6 cc. of the material were introduced into each previously sterilized vial, which was provided with a cotton plug. Usually a sufficient number of vials was prepared at a time to supply requirements for two or three days and kept in a refrigerator while awaiting use. Before introducing a pair of flies into a fresh vial, a small amount of powdered Magic Yeast was dusted on the surface of the culture medium and on this was placed a disc of towel paper cut somewhat smaller than the bore of the vial. When a very limited amount of paper is placed in a vial, the larvæ developing therein pupate on the glass without "spinning" and therefore require a minimum of cleaning in preparation for use. They can then be removed quite readily from the glass without danger of injury with a small brush after a preliminary wetting with water.

Whenever possible, the first 10 pupæ appearing in a vial were used for a determination, but quite frequently only a smaller number could be secured. Each evening the vials for the several matings were examined and any pupa which had appeared unduly early was checked with a wax pencil in order that it

might not be included among the experimental pupæ selected the following morning. Accordingly, the maximum age of pupæ on which determinations were made was 15 or 16 hours, a point which must be kept in mind.

In preparation for the first reading a lot of pupæ was first washed in water with a camels' hair brush, then treated with 80 per cent. alcohol for 2 or 3 minutes to destroy any adhering yeast cells, rinsed in water and dried on filter paper. After being weighed on a delicate balance they were placed in a cotton-lined basket and suspended in the oxygen-measuring apparatus. Between determinations each lot of pupæ was kept in an individual moist chamber with a piece of moist filter paper. Weighings as well as determinations were continued each day until development was so far advanced that there was danger of flies emerging. As a final step, record was made of the sexes of the flies which issued from each lot of pupæ.

Rates of oxygen consumption were measured with an improved form of the manometer of Krogh (1915), described by Bodine and Orr (1925). Six manometers were used, a single one only being used for a given lot of pupæ during the period of pupal life. During readings the manometers were placed in a water bath which was kept at a constant temperature. From day to day, however, the temperature of the bath varied in accord with the temperature of the room, but this fluctuation is not registered in the determinations as oxygen values are always reduced to 0° C. Calculation of rates has been made on the basis of oxygen consumption per minute of time and both per gram body weight and per single pupa.

The "Oxygen Curve."

The duration of pupal life is influenced largely by temperature. An index of this correspondence is afforded by figures for the number of days on which oxygen determinations were possible—even though such figures do not represent the actual duration of pupal life. During the first experimental period 4-day pupæ were predominant, 5-day pupæ occurring rarely and 3-day pupæ only to the extent of 7 per cent. At a temperature of 25° C., on the other hand, the percentage of 3-day pupæ was increased to 60 per cent., while 5-day pupæ did not occur.

During the course of pupal development certain readjustments are in progress which are reflected in rates of metabolism. In Fig. 1 the changes in metabolism are shown in the form of curves. Those for 4-day pupæ are derived from a single mating of the second experimental period while those for 5-day pupæ pertain to the first period. Each curve shows an initial fall after the first day of pupal life succeeded by an abrupt or gradual rise. The most instructive are the curves for 5-day pupæ in which the period of depression of metabolic rate is seen to continue from the second to the third day. The curves for 4-day pupæ are obviously abbreviations of these.

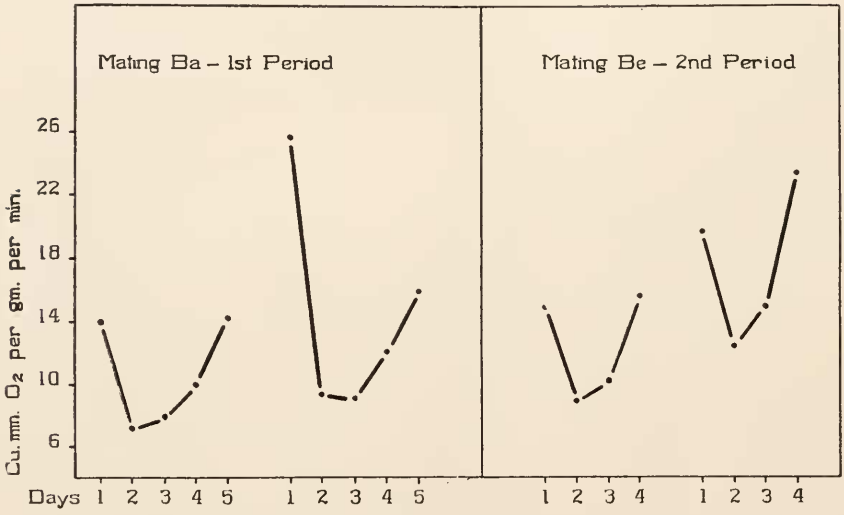

FIG. 1. Metabolism curves based on O_2 consumption per minute per gram of body weight. Ordinates = O_2 values in cubic millimeters; abscissæ = time in days.

This type of curve appears to be characteristic of pupæ in general, the modifications presented in the several species that have been investigated being due chiefly to the varying extensions of the period of depression. The significance of this type of pupal curve has been discussed at length by several workers among whom may be mentioned Tangl (1909, 1 and 2), Weinland (1906), and quite recently Fink (1925). The researches of Weismann, Perez, and others have demonstrated that early in pupal life the persisting larval tissues undergo a series of histolyses

leading to their ultimate dissolution, and that the tissues of the imago are built up through the activity of certain groups of cells which survive histolysis and appear to be set aside for this specific purpose. In other words, two distinct processes are in progress during pupal life—a destructive and a constructive, the latter being inaugurated before the completion of the former. The authors cited identify the abrupt fall in metabolism early in pupal life with the histolytic process and the recovery after the period of depression with the formation and growth of imaginal tissues.

Variability in Rates of Metabolism.

When a series of curves for rates of oxygen consumption is examined, a feature which is most striking is the considerable variability in values exhibited. The majority of the values for any one day of pupal life fall within fairly narrow limits, but scattered among these are numerous others representing very high as well as rather low rates, distributed in a seemingly erratic manner. An early examination of the data proved that most of the very high rates belonged to lots of pupæ, one or more members of which not only failed to give issue to flies but failed to pass beyond the stage of development characteristic of second day pupæ. Instances of this sort suggest that probably intestinal microörganisms find their capacity for growth released by the death of the pupa or pupæ harboring them, and by their rapid multiplication elevate the rate of oxygen consumption to a high level. With one exception to be considered later, it was deemed necessary to completely eliminate from further consideration all data pertaining to lots of pupæ showing incomplete development, thereby reducing the total number of determinations by about one third. Several further eliminations are for lots of pupæ which were accidentally shaken from their supports within the manometers into the 2 per cent. NaOH solution over which they were suspended. Although washed as quickly as possible in a large volume of water and again set up in the manometer, a lot so treated almost invariably responded with an abnormal elevation of rate. Mention should also be made of a series of determinations which was set aside because of bacterial contamination

of the cultures. This condition resulted in the production of a limited number of very small pupæ of exceptionally low weights. A single example from this series has been retained in Table 5 in order to show that, in spite of the very low pupal weights, the rate of metabolism is to all appearances normal. The rather drastic elimination which has been practiced has reduced to a considerable degree both the number of determinations and the variability in rates, but that the latter has been by no means removed is shown in Fig. 2.

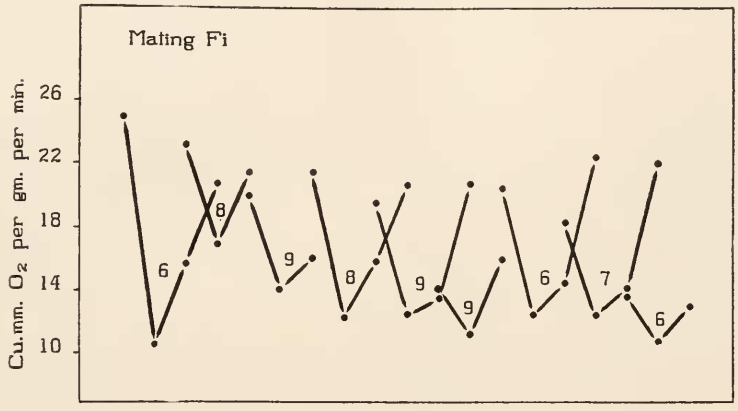

FIG. 2. Metabolism curves showing variability in rates within a single mating, cultured at 25° C. Numbers indicate particular manometers used in establishing rates. Ordinates = O_2 per gram per minute, in cu. mm.

There are several conceivable factors which might underlie this variability and they will be taken up in turn. It is obvious that if comparisons are to be made between the several stocks in respect to oxygen consumption this irregularity must be reduced to a minimum. Suspicion was at once cast on the calibration of the manometers used in making the determinations. That these are not the primary source of the variability will be evident from a further consideration of Fig. 2. The curves of this figure are for lots of pupæ all of which are offspring of a single pair of flies. Associated with each curve is a number which is that of the manometer used in determining the rates. For each of two manometers, numbers 6 and 9, three sets of determinations are represented which, it should be noted, exhibit marked variability in rates of metabolism. The manometers were very carefully

calibrated several times and any possible errors from this source, although carefully looked for, have eluded detection.

Pupal Weights and Metabolism.

In studies on respiratory metabolism the practise has been to emphasize the relation between weights of experimental organisms and intake of oxygen or production of carbon dioxide, on the assumption that for comparable samples of living material within a species, the rates of metabolism remain fairly constant. Rubner, along with others, on the other hand, has instituted the procedure of basing metabolism on extent of body surface, arguing, in the words of Krogh (1916) that "the metabolism is simply a function of the conditions for loss of heat, while there is no such thing as a specific oxidative activity of the cell." Rubner later saw fit, however, to qualify this idea. These practises were derived from investigations on warm-blooded animals possessed of a heat-regulating mechanism, and their applicability to invertebrate animals is extremely uncertain. Unfortunately, the studies on metabolism in invertebrates have been so few in number and have afforded results of so conflicting a character that conclusions based on them do not seem to be justified. We feel obliged, therefore, to examine the data for *Drosophila* with some care and determine, if possible, the significance to be attached to weight; and attempt to decide whether or not rates of metabolism are subject to decided change. No data are available on which to base figures for pupal surface.

Pupal weights exhibit a wide range in value, the means for newly-formed or first-day pupæ in lots of 10 varying between 11 and 15 milligrams. At the extremes of the range lots of 10 pupæ may possess a weight as low as 9 mg. or as high as 17 mg. For the products of a single mating, likewise, the range of variability is very pronounced.

In addition to the rôles played by food and overcrowding as factors affecting pupal weight—and these can hardly be considered as applying in this work—temperature certainly is a determining agency. An examination of Table 2 shows that pupal weights of the second period are significantly lower than those for the first period. In the summer of 1923, during a

Table II.

Pupal Weights and Oxygen per Gram for Total Stocks.

Figures for weights are based on lots of 10 pupæ, stated in milligrams; figures for oxygen consumption are rates per minute, stated in cubic millimeters.

	1st Day.	2d Day.	3d Day.	4th Day.
First Period—4-Day Pupæ.				
Weights	14.04 ± .20	12.57 ± .17	12.46 ± .17	12.36 ± .18
O_2 per Gram	15.89 ± .34	9.00 ± .15	10.38 ± .21	15.44 ± .42
Second Period—4-Day Pupæ.				
Weights	13.30 ± .18	11.87 ± .15	11.78 ± .17	11.65 ± .18
O_2 per Gram	18.35 ± .44	11.00 ± .20	13.30 ± .35	21.15 ± .35
Second Period—3-Day Pupæ.				
Weights	13.45 ± .18	12.25 ± .14	12.06 ± .14	
O_2 per Gram	17.94 ± .38	12.28 ± .28	16.71 ± .36	

period when the room temperature varied between 25° and 30° C., a collection of weights was made which for lots of 10 pupæ present mean values of 11.83 ± .152, 10.86 ± .169 and 11.00 ± .194 for the three days of pupal life. It is, therefore, evident that an inverse relation obtains between temperature and pupal weight. When the range of temperature is not very great,

Table III.

Coefficients of Variation for Pupal Weights and Oxygen per Gram.

The coefficients are based on the figures presented in Table II.

	1st Day.	2d Day.	3d Day.	4th Day.
First Period—4-Day Pupæ.				
Weights	11.2 ± 1.03	10.4 ± .97	10.4 ± .97	10.7 ± 1.02
O_2 per Gram	16.5 ± 1.51	13.0 ± 1.22	15.1 ± 1.41	20.3 ± 1.93
Second Period—4-Day Pupæ.				
Weights	9.8 ± .97	9.1 ± .90	10.3 ± 1.02	10.2 ± 1.09
O_2 per Gram	17.3 ± 1.72	13.1 ± 1.30	18.5 ± 1.84	10.9 ± 1.16
Second Period—3-Day Pupæ.				
Weights	11.3 ± .94	9.9 ± .83	9.4 ± .80	
O_2 per Gram	18.3 ± 1.52	19.2 ± 1.62	17.9 ± 1.53	

however, this correlation does not manifest itself when curves for temperature and pupal weights are compared. Moreover, a constant temperature serves merely to limit somewhat the range of fluctuation, instead of insuring stability, as can be seen from Table III. Temperature, then, operates only in a large way in its influence on weight of pupæ.

Corresponding to the sudden drop in metabolism shortly after the inauguration of pupal life, there occurs a marked drop in pupal weight. Sometimes this loss is relatively enormous, in other exceptional cases relatively insignificant. Unlike metabolism, however, there is no recovery in weight after the second day, the level for the third and fourth days remaining approximately identical with that for the second day. Sometimes for these latter days a slight increase is registered but as frequently a slight fall occurs.

The interesting sequence of changes during the pupal period in respect to pupal weights and metabolism is presented in Fig. 3. The data on which this figure is based represent a mating selected for illustration because a large number of determinations is available and the pupal weights show an unusual consistency of level. The figure is divided into parts corresponding to the several days of pupal life and a common scale is used which enables one to carry out comparisons between any two days.

The relationship between the curves for metabolism per pupa and per unit body weight affords an index of the ratio existing between weight of respiring tissue and that of inert materials associated with the respiring tissue. When the two curves follow a strictly parallel course this ratio must remain constant in the several lots of pupæ, provided complicating factors are not present. When for a particular determination the curves become approximated, the inference may be made that the relative amount of non-respiring or inert materials is reduced, whereas, when they become more widely separated, this condition may be attributed to a relative increase in the non-respiring materials present. It will be clear, therefore, that in general the ratio of respiring to non-respiring substances in the pupæ on which Fig. 3 is established remains a fixed quantity. Interesting exceptions, however, appear in the first and last determinations, numbers 8

and 37. In the latter the relation of the metabolism curves for the first day indicates that the excessive weight of the pupæ in question was due to the presence of a disproportionately great amount of non-respiring material, let us suggest water. As pupal development proceeded, this disproportion gradually became

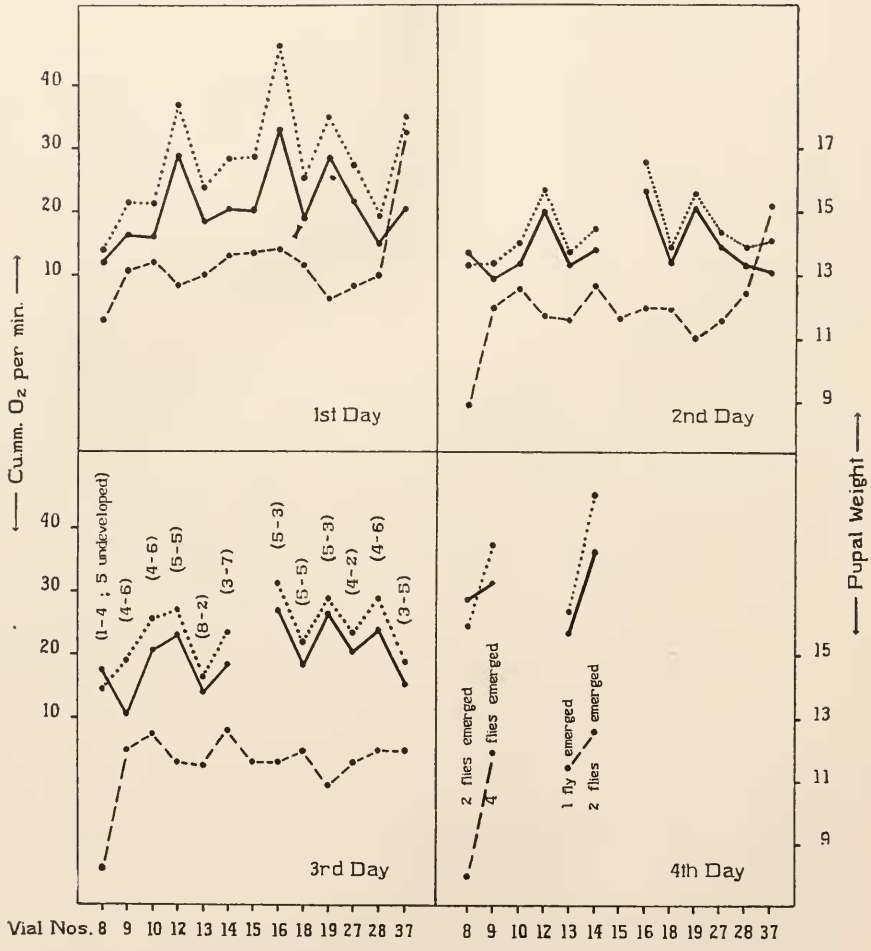

FIG. 3. Modifications in metabolic rates and pupal weights during pupal life for lots of pupæ derived from a single mating, C–2:4, at 25° C. Corresponding values for each day of pupal life are connected into curves. Upper curve = O_2 per pupa; middle curve = O_2 per gram and lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weights in milligrams per 10 pupæ. Abscissæ are numbers of the vials from which experimental pupæ were obtained. Sex-ratios in brackets (males—females).

reduced until, on the third day, the normal balance of respiring and non-respiring materials was restored. The first determination, number 8, on the other hand, presents a different story which is complicated by the fact that 5 of the 10 pupæ on which this determination is based failed to complete their development, the only case of the sort we shall consider. The approximation of the metabolism curves suggests that the pupæ contained a very small amount of non-respiring material relatively to the respiring tissues, yet this idea does not seem to harmonize with the remarkable fall in pupal weight occurring after the first day of pupal life. If, however, we postulate for this case, as was done earlier for cases of arrested development in general, a bacterial activity which would serve to elevate the metabolism per unit body weight relatively to the total metabolism, we develop an interpretation which affords an understanding not only of the peculiar relation shown by the two curves but also of the cause for the unusual decrease in pupal weight. Instances of this sort, representing a fluctuation in the relative amounts of respiring and non-respiring substances present in pupæ, are of frequent occurrence and are further illustrated in Fig. 5. The only general statement that seems permissible is that pupæ of very low weight usually show an elevation of the rate per unit weight relatively to the total metabolism but this condition is by no means invariably true. It appears, therefore, that mere weight is a rather unreliable index of the amount of respiring tissue.

It is manifest that for the first and second days of pupal life a rough proportionality certainly exists between pupal weights and metabolism, thus explaining in a way the trends of the oxygen curves. However, the peculiarities of the 12th, 16th and 19th determinations must receive an interpretation of their own. As the two metabolism curves remain closely parallel, the only conclusion that seems justifiable is that the rates for the determinations in question are of a discontinuous type; in other words, independent of pupal weight. It would appear, therefore, that strictly comparable samples of respiring tissue grown under, and subjected to, like environmental conditions can nevertheless exhibit marked diversity in rates of metabolism. It is ex-

ceedingly improbable that the independent rates of these determinations are due to a secondary source, bacterial for example, contributing in an additive manner to the respiring pupal tissues. If this were the case, we should expect the condition to be indicated by the metabolism curves.

As applied to *Drosophila*, the practice of bringing metabolism into relation with pupal weight is warranted only in a most general way. The correlation is most pronounced during the first day of pupal life when the coefficient of correlation for 4-day pupæ grown at 25° C. attains a value of 0.47 ± .071. On the second and subsequent days this value is considerably reduced. Too much importance should not be attached even to this correlation. The relationship often fails completely in individual cases, as is shown in Fig. 5. This fact, taken in connection with the rather frequent tendency toward the establishment of discontinuous rates, indicates that an understanding of metabolism in *Drosophila* is not to be sought on the basis of weight of respiring tissue but rather through the impress of factors regarding whose nature we are at present in ignorance.

A point of considerable interest brought out in Fig. 3 and in other figures for similarly prepared material is the constancy of the relation between the respiratory rates for the individual lots of pupæ up to the third day of pupal life. The striking similarity of the metabolism curves for the first and second days of pupal life indicates that the rates for this period are relatively stable and that the establishment of independent rates may occur either before or later but hardly during this period. On the third day, however, after the inauguration of growth and differentiation of imaginal tissues, a new order of rates is ushered in, which is that of adult life. As the metabolism curves for the third day in Fig. 3 show, these new rates considered in their entirety still preserve to a slight degree their kinship with those of early pupal life, but each lot of pupæ now develops along a new course. It appears that the rates of first-day pupæ in no way can serve to forecast those of the flies which will later emerge from them. We have already suggested that the period of institution of new adult rates is a somewhat critical stage in pupal development. The great majority of pupæ of arrested

development, apart from those containing fully formed flies unable to escape from the pupal cases, represented the stage of development characteristic of 2-day-old pupæ. In other words, the histolytic process had been gone through but the reconstructive had failed of attainment.

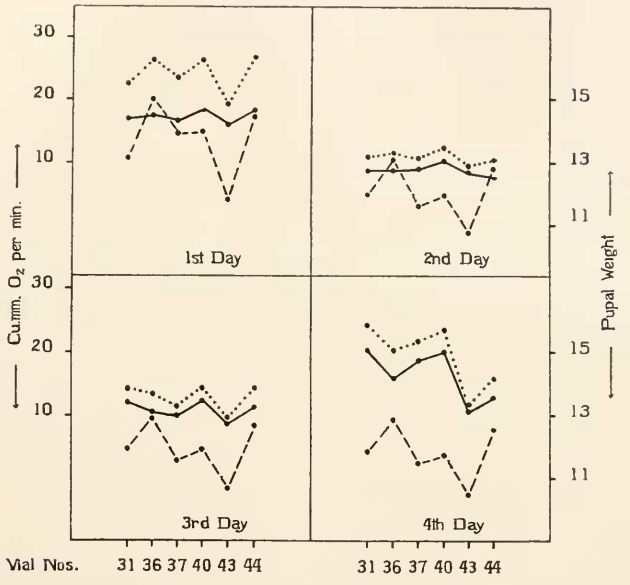

FIG. 4. Metabolic rates and pupal weights for pupæ of the first experimental period, products of single mating Hd. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates = O_2 values in cu. mm. at left, pupal weights in mg. per 10 pupæ at right. Abscissæ = numbers of the vials from which experimental pupæ were obtained.

Do flies about to emerge possess a higher standard metabolism than do larvæ? Our selected figure throws some light on this question. The elevated rates for the four-day pupæ in the figure are certainly in large part to be explained as due to muscular contractions incident to emergence of the flies, and accordingly these records do not indicate standard metabolism. When we recall that the figures for the first day of pupal existence are for pupæ which may be as old as 15 or 16 hours and in which histolysis and its accompanying depression in rate of metabolism has perhaps been in progress for an unknown length of time, it would appear from a comparison of the rates for the first and

third days of pupal development that the standard metabolism of larvæ is actually somewhat greater than that of the flies although, relatively to body weight, the two stages may be more nearly comparable.

Before turning from the general subject we have been considering, a point of difference between the metabolism curves for the first and second experimental periods should be noted. For a cultural temperature of 25° C., we have seen that marked fluctuations occur in rates of oxygen consumption among lots of pupæ derived from a single mating, and corresponding in a very rough way with variations in pupal weight. In material representing the first experimental period at a lower temperature, the extent of fluctuations in metabolism is less pronounced until the last day when it becomes increased. Pupal weights of the first period are more irregular than for the second period and in consequence the parallelism between weight and metabolism tends to become lost. The relationship between pupal weight and metabolism for this period is represented in Fig. 4.

Influence of Sex on Metabolism.

It has been pointed out that for any one day of pupal life the metabolic rates for different samples of pupæ vary considerably and that weight is a very untrustworthy guide in arriving at an understanding of this irregularity. Is sex a contributing factor? Biological literature abounds in references to the physiological distinctness of the sexes and several important recent researches have depended for interpretation on the postulate that male animals possess a higher rate of metabolism than do female. So far as the writer knows, only one experimental attempt has been made to measure this supposed difference directly, namely, the investigation by Benedict and Emmes (1915). With human subjects these workers found a slight increase in metabolism in favor of males over the rates for females.

There are certain facts at hand to suggest a possible difference in metabolism between the sexes of *Drosophila*. At times, at least, there is a well developed tendency for female flies to emerge earlier than the males from the first-formed pupæ. The present data happen to be inconclusive on this point. The

TABLE IV.

SEX AND PUPAL WEIGHTS.

Pupal weights are stated in milligrams for lots of 10 pupæ.

First Day.

♂♂	Pupal Weights.										♀♀
	8	9	10	11	12	13	14	15	16	17	
1											9
2							×	14.30 ± 1.187			8
3							×	14.37 ± 0.780			7
4						×		13.35 ± 1.630			6
5						×		13.90 ± 1.337			5
6					×			12.87 ± 2.152			4
7					×			12.79 ± 1.778			3
											2
9											1

Second Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2						×		12.90 ± 1.300			8
3						×		13.00 ± 0.223			7
4					×			12.08 ± 1.245			6
5					×			12.38 ± 1.132			5
6				×				11.64 ± 1.679			4
7				×				11.58 ± 1.418			3
8											2
9											1

Third Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2											8
3					×			12.66 ± 0.145			7
4					×			12.00 ± 0.107			6
5					×			12.31 ± 0.175			5
6				×				11.54 ± 0.278			4
7				×				11.72 ± 0.213			3
8											2
9											1

records do, however, suggest a tendency for larvæ destined to become females to pupate earlier than those which will develop into males. Also, as is shown in Table IV., pupæ which are to become females incline toward heavier weight than those which become males.

The sex ratios for a typical series of determinations have been indicated in Fig. 5 as well as in Fig. 3. All of the data have

been gone over most carefully with the unqualified result that sexual differences in metabolism are impossible to detect. If such exist they must be so small that they are obscured by irregularities induced by other agencies.

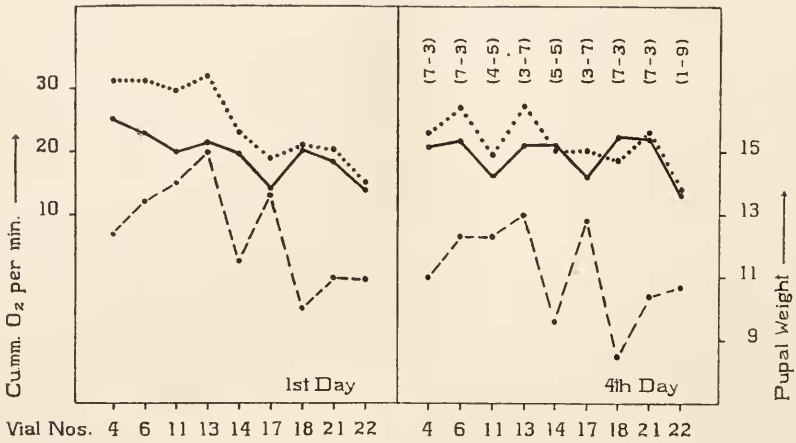

FIG. 5. Oxygen rates, pupal weights and sex-ratios for lots of pupæ of mating Fi, of the second experimental period. Curves for the second and third days of pupal life are omitted. Sex-ratios shown in brackets, the first figure for males, the second for females. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weight values in mg. per 10 pupæ. Abscissæ refer to the vials from which experimental pupæ were obtained.

FURTHER CONSIDERATIONS ON IRREGULARITY IN RESPIRATORY RATES.

The most significant result which this study has revealed is the peculiar irregularity in rates of metabolism exhibited especially by pupæ formed under conditions of constant temperature. It might be remarked that there is no ground for believing that the causes underlying this irregularity are genetic in character. On the contrary, the usual tendency of the rates for lots of pupæ derived on consecutive days from a common source to exhibit a graduated character either in the direction of elevation or of depression strongly suggests the effect of graduated environmental influences. We have already eliminated pupal weight and sex as important agencies responsible for irregularity in rates and we may now refer to several remaining possibilities.

It is believed that under the conditions of the experiment food conditions were maintained as nearly uniform as is possible. Moulds occasionally became established in the culture vials but the growth rarely if ever became noticeable until after the larvæ had pupated. It is altogether possible that organisms harmful to the larvæ and of sporadic occurrence in the tubes may have contributed in slight measure to the irregularity, but this is hardly a likelihood. We have referred to the bacterial contamination of one series of cultures, with the consequent reduction in weight of the pupæ. Curiously enough, this reduction in weight does not appear to have induced a parallel modification in rate of metabolism.

The pupæ of *Drosophila*, it would seem, are adapted to resist conditions of desiccation, at any rate for short periods. On several occasions lots of pupæ were inadvertently subjected overnight to the drying effect of the room air. This treatment produced no detectable effect on the following day either in pupal weight or in rate of oxygen consumption. This point is of some interest in connection with the results obtained by Caldwell (1925), who has found that the metabolism of some animals, as measured by carbon dioxide production, is modified as a result of desiccation.

A degree of irregularity in oxygen rates was certainly introduced with varying ages of the pupæ used. Larvæ developing from eggs deposited on a single day possess individual peculiarities, some pupating precociously, others delaying the act for a day or longer after their brothers and sisters have made the change. Accordingly, all of the pupæ thrown together for a determination are not of the same age. Among lots of experimental pupæ, however, the mean ages should be nearly identical as selection was performed at about the same hour each morning and the manometer readings were distributed over approximately identical time periods each day.

Mention might be made of an attempt to find an interpretation of the irregularity in rates through a study of varying intensities of such environmental factors as light, humidity, etc., data for which were secured from the local weather office. As might be expected, this attempt was not fruitful of results. Likewise, a

careful examination of data on length of larval life, of pupal life, etc., proved barren of results, except that a suggestive but very rough correspondence between rates of oxygen consumption and productivity, as measured by the number of flies appearing in the vials, did come to light. A correspondence of this sort would carry the implication that the metabolic rates of the pupæ are due to an impress set by the metabolic condition of the female parent at or about the particular time the eggs are deposited, and that this impress continues without impairment over the period of larval life. The data at hand do little more than suggest this possibility.

Effects of Inbreeding.

The central question around which this study was planned was whether or not it is possible to find an index of inbreeding in rates of metabolism. If metabolism were subject to control as a result of genetic make-up, we should expect to find evidence of it reflected in our results. There is not only no indication that heredity plays any but the most general rôle in metabolism; but, on the contrary, we have seen some reason for believing that rates are at the command of graduated environmental influences. The irregularity in rates which we have stressed renders it impossible to make satisfactory comparisons between different matings within a common stock, and the propriety of lumping all of the matings of a stock under a mean is very questionable. However, this has been done in Table 5 in which are presented means for most of the stocks. The eliminations previously referred to made such serious inroads on the data for the majority of the matings that Table V. must be built up on an inadequate number of determinations. The figures for stock G of the first period are based on as few as 3 determinations; and in all other cases on 4 determinations except where a probable error is attached, this indicating that 5 or more determinations were available. Deficient as it is, this table illustrates the impossibility of separating inbred from non-inbred stocks on the basis of rates of metabolism.

Summary.

Several results emerge from this study which, it is believed, should assist in defining the problem and, at the same time,

TABLE V.

PUPAL WEIGHTS AND OXYGEN PER GRAM (IN CU. MM. PER MINUTE) FOR INDIVIDUAL STOCKS.
Non-inbred stocks are starred (*).

Stocks.	1st Day.		2d Day.		3d Day.		4th Day.	
	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.
FIRST PERIOD—4-DAY PUPÆ.								
H*	14.25 ± .31	17.12 ± .42	12.62 ± .16	9.50 ± .18	12.62 ± .16	10.62 ± .29	12.62 ± .16	15.75 ± .73
G*	13.00	11.50	12.50	8.50	12.50	11.00	12.50	15.50
P	15.40 ± .15	15.20 ± .48	13.80 ± .12	8.60 ± .31	13.40 ± .24	10.20 ± .22	13.25	15.70
I	14.33	15.25	12.66	8.00	12.66	9.00	12.33	12.66
C–2	12.20 ± .35	16.40 ± .84	10.50	9.50	10.25	10.70	10.25	15.20
C–1	14.66	17.33	13.00	9.66	13.00	11.00	13.00	17.33
SECOND PERIOD—4-DAY PUPÆ.								
H* [1]	11.71	17.42	10.71	9.71	10.40	11.75	10.66	17.00
G*	13.75	17.50	12.50	10.00	12.25	12.50	12.25	20.50
I	14.25	14.75	12.25	9.25	12.25	11.50	12.25	18.25
C–2	13.60 ± .15	19.60 ± .67	12.20 ± .12	12.00 ± .38	12.20 ± .12	14.00 ± .63	12.00	23.50
F	12.60 ± .33	19.30 ± .74	11.30 ± .28	11.60 ± .24	11.20 ± .34	14.60 ± .46	11.10 ± .31	22.10 ± .29

[1] The low weights of the pupæ of stock H of the second period were due to pollution of the cultures by bacteria.

should suggest types of investigation which are most likely to contribute to an understanding of metabolism in *Drosophila melanogaster*.

It has been shown that a close approach to a knowledge of metabolism in *Drosophila* pupæ cannot be made if sole dependence is placed on weight of respiring tissues as a guide. In a very rough way, pupal weights show a correspondence with the trends of metabolic rates, but, for particular matings, especially when the larvæ are maintained at lower temperatures, the correspondence is largely lost. It has also been shown that rates of oxygen consumption are very irregular and vary in a manner highly suggestive of the influence of environmental agencies. The not infrequently pronounced elevations above the general level of metabolism lend support to the conclusion that the metabolic rates for comparable samples of respiring tissue are not necessarily fixed within narrow limits. Finally, it has proved impossible to find a metabolic difference between the sexes or to detect any difference in metabolism between inbred and non-inbred stocks.

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